



# SUBSTITUTE SHEET 1 of 14

## SEQUENCE LISTING

Murdoch University  
Novartis Animal Health US, Inc.  
Hampson, David John  
La, Tom

<120> Brachyspira pilosicoli 72 kDa outer membrane protein and diagnostic and therapeutic uses thereof.

<130> 34141A

<140> US 10/583,202

<141> 2006-06-16

<150> PCT/AU2004/001783

<151> 2004-12-17

<150> AU2003907017

<151> 2003-12-19

<160> 39

<170> PatentIn version 3.5

<210> 1

<211> 1750

<212> DNA

<213> Brachyspira pilosicoli

<400> 1

atgagtactt taataaaagaa aatcgtagct tatatacgctt taatctcttt tagtttttagc 60

gtattacctg ctcaaactta tggatgtcg gctagaatta ctggagaagc tgagacttta 120

caaaaatgacg gagaataccaa aaagtcttat gataaaatctc aagaggcttc tgactctata 180

gataaaaacta ctgtatcatt attttataga ttaatgaact taagaatagc taaagcaaaa 240

aatgatgcaa ataagactat taatgaaata gaacaattag gtgcattcac tgataatgaa 300

tttaaaacaa aatatcaaga agctctaaaa ttctttgaag aaggaaataa tagtattact 360

aacttacctc cagaaccgca aactccctc acagatgaag agtttactgc ttcttcaaacc 420

acattcacta cagtatataa ttctttcaac aatgcatttac aatctgctaa cagtgaaaa 480

gaaggttatac ttaatagaga aagagcaata gcttcaaaat ccattaatga tgctagaaac 540

aaatataaaag cagaattagg caagagtgtt aagcaggcg atgctaatttga tagaaatatt 600

aatggtgctt taacttagagc tggatgtcg ctttagcaatg acaattttgc aagcgttcag 660

cagaatgtat ctactgcatt agctggata aataaagcta tagcagatgc taaggcgaaa 720

gctgaggcg aagctaaagc aaaagctgct gctgaagcta aggcaagagc tgaagcagag 780

gctaaagcga aagcagaagc tgctgctaaa gcaaaagctg aagcagaggc taaagcgaaa 840

gcagatgcaa tagcaaaagc taaaaaagac atagaagatg cacaaaataa atataataat 900

## SUBSTITUTE SHEET 2 of 14

ttagttaatg atcaagtaat agctaaaggat gatgataatg ataaaaacgt atcaaaactt	960
ttaactgatg ctaataatgc tttacaaaac actcctcaaa ctgcaagcga taaagcttta	1020
gaagcttcta aaactatgga taatatatta aacactgcta atcaattgaa aaaagaagaa	1080
gctgttaaaa atctagagca attaaaggca agaagagaca gacttataag cgaaggttat	1140
ttaactaaag acagcgaaga agaacaaaag ttatctcaaa ctattaaaga agctgaagat	1200
gctttaata acaatgatta tgtttagct gaccaaaaaa tgcaggaagc taatcttaac	1260
atgaatgcta tagaagagag aggacctatt gacggacaag ttatacctgg tgaaatggc	1320
ggtaacgaaa ctggtcaaatt aattgatgct actactggtc aagaagtaaa tacagaagga	1380
aaagttaactg tattacctca atattatgtt gtagtaagaa gagtacctct aactgatgct	1440
ttatggagaa ttgctggata cagctacata tacaacaacc ctatagaatg gtacagaata	1500
tatgaagcta acagaaatgt acttagagac cctaataacc ctgatttaat acttcctgg	1560
caaagattaa taatacctag ccttaatggta gaagagagaa gcggtgatta taatcctgat	1620
ttagagtatt tgacttatga tgaggttagt cagtttaagac agcaaaataa cactactcaa	1680
gcacaacagt aagaaataaa cttataaaat acaaaaggc atgcatttaa tatgtatgac	1740
cttttttgt	1750

<210> 2  
 <211> 563  
 <212> PRT  
 <213> **Brachyspira pilosicoli**

<400> 2

Met Ser Thr Leu Ile Lys Lys Ile Val Ala Tyr Ile Ala Leu Ile Ser			
1	5	10	15

Phe Ser Phe Ser Val Leu Pro Ala Gln Thr Tyr Asp Asp Ala Ala Arg		
20	25	30

Ile Thr Gly Glu Ala Glu Thr Leu Gln Asn Asp Gly Glu Tyr Gln Lys		
35	40	45

Ser Tyr Asp Lys Ser Gln Glu Ala Ser Asp Ser Ile Asp Lys Thr Thr		
50	55	60

Val Ser Leu Phe Tyr Arg Leu Met Asn Leu Arg Ile Ala Lys Ala Lys			
65	70	75	80

Asn Asp Ala Asn Lys Thr Ile Asn Glu Ile Glu Gln Leu Gly Ala Ser		
85	90	95

SUBSTITUTE SHEET 3 of 14

Thr Asp Asn Glu Phe Lys Thr Lys Tyr Gln Glu Ala Leu Lys Phe Phe  
100 105 110

Glu Glu Gly Asn Asn Ser Ile Thr Asn Leu Pro Pro Glu Pro Gln Thr  
115 120 125

Pro Pro Thr Asp Glu Glu Phe Thr Ala Ser Ser Asn Thr Phe Thr Thr  
130 135 140

Val Tyr Asn Ser Phe Asn Asn Ala Leu Gln Ser Ala Asn Ser Val Lys  
145 150 155 160

Glu Gly Tyr Leu Asn Arg Glu Arg Ala Ile Ala Ser Lys Ser Ile Asn  
165 170 175

Asp Ala Arg Asn Lys Tyr Lys Ala Glu Leu Gly Lys Ser Val Lys Ala  
180 185 190

Gly Asp Ala Asn Asp Arg Asn Ile Asn Gly Ala Leu Thr Arg Ala Asp  
195 200 205

Glu Ala Leu Ser Asn Asp Asn Phe Ala Ser Val Gln Gln Asn Val Ser  
210 215 220

Thr Ala Leu Ala Gly Ile Asn Lys Ala Ile Ala Asp Ala Lys Ala Lys  
225 230 235 240

Ala Glu Ala Glu Ala Lys Ala Lys Ala Ala Ala Glu Ala Lys Ala Arg  
245 250 255

Ala Glu Ala Glu Ala Lys Ala Lys Ala Glu Ala Ala Ala Lys Ala Lys  
260 265 270

Ala Glu Ala Glu Ala Lys Ala Lys Ala Asp Ala Ile Ala Lys Ala Lys  
275 280 285

Lys Asp Ile Glu Asp Ala Gln Asn Lys Tyr Asn Asn Leu Val Asn Asp  
290 295 300

Gln Val Ile Ala Lys Gly Asp Asp Asn Asp Lys Asn Val Ser Lys Leu  
305 310 315 320

Leu Thr Asp Ala Asn Asn Ala Leu Gln Asn Thr Pro Gln Thr Ala Ser  
325 330 335

SUBSTITUTE SHEET 4 of 14

Asp Lys Ala Leu Glu Ala Ser Lys Thr Met Asp Asn Ile Leu Asn Thr  
340 345 350

Ala Asn Gln Leu Lys Lys Glu Glu Ala Val Lys Asn Leu Glu Gln Leu  
355 360 365

Lys Ala Arg Arg Asp Arg Leu Ile Ser Glu Gly Tyr Leu Thr Lys Asp  
370 375 380

Ser Glu Glu Glu Gln Lys Leu Ser Gln Thr Ile Lys Glu Ala Glu Asp  
385 390 395 400

Ala Leu Asn Asn Asn Asp Tyr Val Leu Ala Asp Gln Lys Met Gln Glu  
405 410 415

Ala Asn Leu Asn Met Asn Ala Ile Glu Glu Arg Gly Pro Ile Asp Gly  
420 425 430

Gln Val Ile Pro Gly Glu Met Gly Gly Asn Glu Thr Gly Gln Ile Ile  
435 440 445

Asp Ala Thr Thr Gly Gln Glu Val Asn Thr Glu Gly Lys Val Thr Val  
450 455 460

Leu Pro Gln Tyr Tyr Val Val Val Arg Arg Val Pro Leu Thr Asp Ala  
465 470 475 480

Leu Trp Arg Ile Ala Gly Tyr Ser Tyr Ile Tyr Asn Asn Pro Ile Glu  
485 490 495

Trp Tyr Arg Ile Tyr Glu Ala Asn Arg Asn Val Leu Arg Asp Pro Asn  
500 505 510

Asn Pro Asp Leu Ile Leu Pro Gly Gln Arg Leu Ile Ile Pro Ser Leu  
515 520 525

Asn Gly Glu Glu Arg Ser Gly Asp Tyr Asn Pro Asp Leu Glu Tyr Leu  
530 535 540

Thr Tyr Asp Glu Val Met Gln Leu Arg Gln Gln Asn Asn Thr Thr Gln  
545 550 555 560

Ala Gln Gln

SUBSTITUTE SHEET 5 of 14

<211> 32  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 3

Lys Val Thr Val Leu Pro Gln Tyr Tyr Val Val Val Arg Arg Val Pro  
1 5 10 15

Leu Thr Asp Ala Leu Trp Arg Ile Ala Gly Tyr Ser Tyr Ile Tyr Asn  
20 25 30

<210> 4  
<211> 22  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 4

Leu Ile Lys Lys Ile Val Ala Tyr Ile Ala Leu Ile Ser Phe Ser Phe  
1 5 10 15

Ser Val Leu Pro Ala Gln  
20

<210> 5  
<211> 13  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 5

Lys Thr Thr Val Ser Leu Phe Tyr Arg Leu Met Asn Leu  
1 5 10

<210> 6  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

SUBSTITUTE SHEET 6 of 14

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 6

Asn Asp Gln Val Ile Ala Lys  
1 5

<210> 7  
<211> 14  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 7

Asp Leu Ile Leu Pro Gly Gln Arg Leu Ile Ile Pro Ser Leu  
1 5 10

<210> 8  
<211> 8  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 8

Asn Asp Tyr Val Ala Leu Asp Gln  
1 5

<210> 9  
<211> 18  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 9

Phe Ala Ser Val Gln Gln Asn Val Ser Thr Ala Leu Ala Gly Ile Asn  
1 5 10 15

Lys Ala

SUBSTITUTE SHEET 7 of 14

<210> 10  
<211> 6  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 10

Val Ser Lys Leu Leu Thr  
1 5

<210> 11  
<211> 13  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 11

Asp Leu Glu Tyr Leu Thr Tyr Asp Glu Val Met Gln Leu  
1 5 10

<210> 12  
<211> 8  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 12

Asn Ala Leu Gln Ser Ala Asn Ser  
1 5

<210> 13  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 13

SUBSTITUTE SHEET 8 of 14

Asp Gly Gln Val Ile Pro Gly  
1 5

<210> 14  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 14

Val Lys Asn Leu Glu Gln Leu  
1 5

<210> 15  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 15

Gly Lys Ser Val Lys Ala Gly  
1 5

<210> 16  
<211> 6  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 16

Gln Glu Ala Leu Lys Phe  
1 5

<210> 17  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

SUBSTITUTE SHEET 9 of 14

<400> 17

Phe Thr Thr Val Tyr Asn Ser  
1 5

<210> 18  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 18

Asn Leu Pro Pro Glu Pro Gln  
1 5

<210> 19  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 19

Lys Ala Asp Ala Ile Ala Lys  
1 5

<210> 20  
<211> 9  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 20

Lys Ala Glu Ala Ala Ala Lys Ala Lys  
1 5

<210> 21  
<211> 7  
<212> PRT  
<213> Brachyspira pilosicoli

SUBSTITUTE SHEET 10 of 14

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 21

Lys Ala Lys Ala Ala Ala Glu  
1 5

<210> 22  
<211> 6  
<212> PRT  
<213> Brachyspira pilosicoli

<220>  
<221> misc\_feature  
<223> mp-72 protein fragment

<400> 22

Asp Lys Ala Leu Glu Ala  
1 5

<210> 23  
<211> 20  
<212> DNA  
<213> T3 phage

<400> 23  
taaccctcac taaaagggaac

20

<210> 24  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> AHP-F1 primer

<400> 24  
tgaatgctat agaagagaga ggac

24

<210> 25  
<211> 22  
<212> DNA  
<213> T7 phage

<400> 25  
gtaatacgac tcactatagg gc

22

<210> 26  
<211> 23  
<212> DNA  
<213> Artificial Sequence

SUBSTITUTE SHEET 11 of 14

<220>  
<223> pTrcHis-F primer  
  
<400> 26  
caatttatca gacaatctgt gtg 23  
  
<210> 27  
<211> 21  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> AHP-Rev primer  
  
<400> 27  
tcgcggcag tttgaggagt g 21  
  
<210> 28  
<211> 21  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> AHP-Rev2 primer  
  
<400> 28  
tggatttga agctattgct c 21  
  
<210> 29  
<211> 22  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> AHP-F4 primer  
  
<400> 29  
caagtaatacg ctaaaggta tg 22  
  
<210> 30  
<211> 22  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> AHP-R783 primer  
  
<400> 30  
ttactgttgt gcttgagtag tg 22  
  
<210> 31  
<211> 24  
<212> DNA  
<213> Artificial Sequence  
  
<220>

SUBSTITUTE SHEET 12 of 14

<223> AHP-98F primer

<400> 31

cgtttagctg aacttgaagc tatg

24

<210> 32

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> AHP+1890R primer

<400> 32

gtaatgctct gtcttaatca t

21

<210> 33

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> AHP+1012R primer

<400> 33

tatcgcttgc agtttgagga g

21

<210> 34

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> AHP-F1-Xho1 primer

<400> 34

agactcgaga gtactttaat aaagaaaaatc gtag

34

<210> 35

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> AHP-R783-EcoR1 primer

<400> 35

gttgaattct tactgttgtg cttgagtagt g

31

<210> 36

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> AHP-R223-EcoR1 primer

**SUBSTITUTE SHEET 13 of 14**

<400> 36		
taagaattcc ttataagtct gtctcttctt g		31
<210> 37		
<211> 31		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> AHP-F4-Xho1 primer		
<400> 37		
ctactcgagc aagtaatgc taaagggtgat g		31
<210> 38		
<211> 23		
<212> DNA		
<213> Artificial Sequence		
<220>		
<223> pTrcHis-R primer		
<400> 38		
tgcctggcag ttccctactc tcg		23
<210> 39		
<211> 1850		
<212> DNA		
<213> Brachyspira pilosicoli		
<400> 39		
agcgtttagc tgaacttgaa gctatggtaa aagagcttga aacttttagag caagagcaac		60
aaagctaata ttttaatatt ttaaggagta taaaagaaaa atgagtactt taataaagaa		120
aatcgtagct tatatagctt taatctctt tagtttagc gtattacctg ctcaaactta		180
tgatgatgcg gctagaatta ctggagaagc tgagacttta caaaaatgacg gagaatacc		240
aaagtcttat gataaatctc aagaggcttc tgactctata gataaaaacta ctgtatcatt		300
attttataga ttaatgaact taagaatagc taaaagcaaaa aatgatgcaa ataagactat		360
taatgaaata gaacaattag gtgcttctac tgataatgaa tttaaaacaa aatatcaaga		420
agctctaaaa ttcttgaag aaggaaataa tagtattact aacttacctc cagaaccgca		480
aactccctc acagatgaag agtttactgc ttcttcaaac acattcacta cagtatataa		540
ttcttcaac aatgctttac aatctgctaa cagtgtaaaa gaaggttatc ttaatagaga		600
aagagcaata gcttcaaaat ccattaatga tgctagaaac aaatataaag cagaattagg		660
caagagtgtta aagcaggcg atgctaattga tagaaatatt aatggtgctt taactagagc		720
tgtgaagca cttagcaatg acaattttgc aagcgttcag cagaatgtat ctactgcatt		780

**SUBSTITUTE SHEET 14 of 14**

agctggtata aataaagcta tagcagatgc taaggcgaaa gctgaggcag aagctaaagc	840
aaaagctgct gctgaagcta aggcaagagc tgaagcagag gctaaagcga aagcagaagc	900
tgctgctaaa gcaaaagctg aagcagaggc taaagcgaaa gcagatgcaa tagcaaaagc	960
taaaaaagac atagaagatg cacaaaataa atataataat ttagttaatg atcaagtaat	1020
agctaaaggt gatgataatg ataaaaacgt atcaaaaactt ttaactgatg ctaataatgc	1080
tttacaaaac actcctcaaa ctgcaagcga taaagctta gaagcttcta aaactatgga	1140
taatatatta aacactgcta atcaattgaa aaaagaagaa gctgttaaaa atctagagca	1200
ataaaaggca agaagagaca gacttataag cgaaggttat ttaactaaag acagcgaaga	1260
agaacaaaag ttatctcaaa ctattaaaga agctgaagat gctttaata acaatgatta	1320
tgttttagct gaccaaaaaa tgcaggaagc taatcttaac atgaatgcta tagaagagag	1380
aggacctatt gacggacaag ttataccctgg tgaaatgggc ggtaacgaaa ctggtcaaatt	1440
aattgatgct actactggtc aagaagtaaa tacagaagga aaagttactg tattacctca	1500
atattatgtt gtagtaagaa gagtacctct aactgatgct ttatggagaa ttgctggata	1560
cagctacata tacaacaacc ctatagaatg gtacagaata tatgaagcta acagaaatgt	1620
acttagagac cctaataacc ctgatttaat acttcctggt caaagattaa taatacctag	1680
ccttaatggt gaagagagaa gcggtgatta taatcctgat ttagagtatt tgacttatga	1740
tgaggttatg cagttaagac agcaaaataa cactactcaa gcacaacagt aagaataaaa	1800
cttataaaat acaaaaggc atgcattaa tatgtatgac ctttttttgt	1850